DRAFT CERHR EVALUATION
of
BUTYL BENZYL PHTHALATE

June 9, 2000

Butyl Benzyl Phthalate

1.0 EXPOSURE 1
1.1 Chemistry 1
1.2 Exposure 1
2.0 GENERAL TOXICOLOGICAL AND BIOLOGICAL PARAMETERS 3
2.1 General Toxicity 3
2.1.1 Human Data 3
2.1.2 Experimental Animal Data 3
2.2 Toxicokinetics 7
2.3 Genetic Toxicity 8
3.0 DEVELOPMENTAL TOXICITY DATA 8
3.1 Human Data 8
3.2 Experimental Animal Toxicity 8
3.2.1 Prenatal Development 8
3.2.2 Postnatal Development 12
3.2.3 Postnatal Function 12
4.0 REPRODUCTIVE TOXICITY 13
4.1 Human Data 13
4.2 Experimental Animal Toxicity 14
5.0 DATA SUMMARY & INTEGRATION 17
5.1 Summary 17
5.1.1 Human Exposure 17
5.1.2 General Biological and Toxicological Data 17
5.1.3 Developmental Toxicity 19
5.1.4 Reproductive Toxicity 22
5.2 Integrated Evaluation 24
6.0 REFERENCES 25

1.0 EXPOSURE

1.1 Chemistry

Butyl benzyl phthalate (BBP) (CAS number 85-68-7) is produced by sequentially reacting butanol and benzyl chloride with phthalic anhydride [CMA, 1999 #825].

Table 1: Physicochemical Properties of BBP

Property	Value
Chemical Formula	$C_{19}H_{20}O_4$
Molecular Weight	312.35
Vapor Pressure	6 x 10^{-7} mmHg at 25 ^oC
Melting Point	-40.5 ^oC
Boiling Point	370 ^oC
Specific Gravity	1.12
Solubility in Water	slight – 2.7 mg/L
Log K_{ow}	4.59

[CMA, 1999 #825]

1.2 Exposure

According to the CMA [CMA, 1999 #825], the largest use of BBP is in vinyl tile. BBP is also used as a plasticizer in PVC used to manufacture food conveyor belts, carpet tile, artificial leather, tarps, automotive trim, weather stripping, traffic cones, and toys, and is used to a limited extent in vinyl gloves. BBP is also used in some adhesives. BBP may be released to the environment during its production and also during incorporation into plastics or adhesives. Because BBP is not bound to the final product, it can be released during the use or disposal of the product. Phthalates that are released to the environment can be deposited on or taken up by crops that are intended for human or livestock consumption, and thus, can enter the food supply.

General Population Exposure

General population exposure to BBP through food has been estimated by at least two authoritative sources: the International Program on Chemical Safety [IPCS, 1999 #1023] and the UK Ministry of Agriculture, Fisheries, and Food [MAFF, 1998 #885; MAFF, 1996 #886; MAFF, 1996 #884].

DBP may enter food by environmental uptake during crop cultivation or by migration from processing equipment or packaging materials. IPCS [IPCS, 1999 #1023] concluded that BBP exposure to the general population is based almost entirely on food intake; they based these food exposure estimates on a survey of 100 food items that were purchased in four Ontario, Canada supermarkets between 1985 and 1988. BBP was only found in yogurt (0.6 μg/g), cheddar cheese (1.6 μg/g), butter (0.64 μg/g), and crackers (0.48 μg/g). Assumptions used to estimate exposure included a 70 kg body weight, and a daily consumption of 13.61 g butter, 3.81 g cheddar cheese, 1.54 g yogurt, 22.73 g pork, and 3.45 g crackers. Adult BBP intake was

estimated at 2 μg/kg bw/day and it was stated that exposure to infants and children could be up to 3-fold higher.

MAFF [MAFF, 1996 #884] estimated adult BBP exposure through dietary intake based on a 1993 survey of fatty foods in the United Kingdom. BBP was detected in carcass meat (0.09 μg/g), poultry (0.03 μg/g), eggs (0.09 μg/g), and milk (0.002 μg/g). In calculating dietary food exposures, MAFF assumed that these types of food likely account for 85% of dietary phthalate intake. Food intake levels were obtained from the Dietary and Nutritional Study of British Adults, but the values were not reported by MAFF. Mean and high level BBP intakes were estimated at 8 μg/person/day and 20 μg/person/day, respectively. Specific details describing the calculations and assumptions used were not provided. Using the IPCS-assumed adult body weight of 70 kg [IPCS, 1999 #1023], the exposure values were converted to 0.11–0.29 μg/kg bw/day.

MAFF also addressed BBP exposure in infants resulting from the consumption of infant formula. A survey published in 1996 reported BBP levels of <0.0044–0.24 μg/g in infant formulas purchased in the UK while a later survey reported BBP levels of <0.003–0.015 μg/g [MAFF, 1996 #886; MAFF, 1998 #913]. It is speculated that the drop in BBP concentration occurred because infant formula manufacturers were urged to reduce phthalate levels after the MAFF published the results of the 1996 survey [MAFF, 1998 #913]. Based on the results from the 1998 survey using an assumed bodyweight of 2.5–3.5 kg at birth and 7.5 kg at 6 months of age, exposure levels were estimated for infants. Formula intake rates were determined from manufacturer instructions. Exposure levels for infants were estimated at 0.2 μg/kg bw/day at birth and 0.1 μg/kg bw/day at 6 months of age. Infants in the United States are likely exposed to lower levels of BBP through formula. In a survey of infant formulas conducted in 1996, BBP levels were below the detection limit of 0.005 μg/g [DHHS, 1996 #903].

BBP was only detected in one sample (2.8 μg/L) collected in 1991in a survey of 300 drinking water sites in two Canadian provinces from1985–1994. IPCS [IPCS, 1999 #1023] considered exposure to BBP through drinking water negligible, exposure through soil intake was also considered negligible.

Mouthing of toys is a potential source of oral phthalate exposure in children. However, use of BBP in toys appears to be rare. In an analysis of 17 plastic toys, BBP was only detected in a PVC doll's head at 0.02% by weight [Rastogi, 1998 #776].

Off-gassing from building materials has been reported to be a potential source of BBP exposure through inhalation; however, exposure has been postulated to be minimal because of the low vapor pressure of BBP. The available data, though minimal, support this view. IPCS [IPCS, 1999 #1023] reported that median air levels of 0.034–0.035 $\mu g/m^3$ were measured in a survey of 125 California homes. BBP levels in outdoor air were also measured for 65 of these homes and the median BBP level was below the detection limit of 0.051 $\mu g/m^3$. The 90^{th} percentile levels of BBP in outdoor air ranged from 5.3–6.7 $\mu g/m^3$ for daytime to evening. IPCS [IPCS, 1999 #1023] considered BBP exposure through inhalation to be negligible.

Dermal contact with products containing BBP is possible, but absorption through skin is most likely minimal. Studies in rats have demonstrated that absorption of BBP through skin is fairly slow (approximately 27% in 7 days) [Elsisi, 1989 #126]. An *in vitro* study conducted with rat and human skin has demonstrated that permeability of human skin to other phthalates (DBP and DEHP) is much lower than that of rat skin [Scott, 1987 #644].

Caution is required in the interpretation of exposure levels for the general population. The exposure estimates by IPCS and MAFF differed by about an order of magnitude. The basis for discrepancies in dietary exposure estimates is difficult to determine for several reasons including: use of different food types

in calculations (e.g., fatty foods vs a variety of foods); use of different assumptions in calculations; varying BBP levels in foods from different countries; and changing BBP levels in food over time. Dietary intake can vary widely depending on the types of foods eaten and the types of materials in which the foods are packaged. It is noted that the food levels reported by MAFF were collected 12–15 years ago and may not reflect current exposure levels.

Medical Exposure

BBP is not known to be used in medical products.

Occupational

Exposure in occupational settings can occur through skin contact and by inhalation of vapors and dusts.

Phthalates are manufactured within closed systems but exposure to workers can occur during filtering or loading/unloading of tank cars [CMA, 1999 #825]. Higher exposures to phthalates can occur during the incorporation of the phthalate into the final product if the process is run at a higher temperature than used in the manufacturing process. The CMA has estimated exposure to BBP in the workplace based upon an assumed level of 1 mg/m^3 during the production of phthalates. An exposure level was estimated by using assumptions of a 10 m^3/day inhalation rate and a 70 kg body weight. The resulting exposure estimate was 143 µg/kg bw/day for workers employed in phthalate manufacturing operations. If the total number of days worked per year is assumed to be 220 days, the exposure estimates converts to 86 µg/kg bw/day. As stated in the General Exposure Section, absorption of BBP through skin is expected to be minimal.

2.0 GENERAL TOXICOLOGICAL AND BIOLOGICAL PARAMETERS

2.1 General Toxicity

2.1.1 Human Data

There are no human data on the general toxicity of BBP. BBP was not observed to be a primary irritant or sensitizer in skin patch tests with volunteers [IPCS, 1999 #1023].

2.1.2 Experimental Animal Data

Multiple studies in mice and rats are available describing the acute, sub-chronic, and chronic toxicity of BBP. These studies assess oral as well as inhalation routes of exposure. There is a study in dogs that examined toxicity in a 90-day feeding study that includes effects that may be related to decreased food consumption.

Acute Toxicity

Acute toxicity of BBP is low, which is evidenced by levels expressed in grams per kilogram. An oral LD_{50} value for BBP in rats is reported as 2–20 g/kg [IPCS, 1999 #1023]. Rabbit dermal and ocular studies revealed no significant concern for BBP-induced sensitization or irritation [Hammond, 1987 #229].

Sub-chronic Studies

Agarwal et al. [, 1985 #10] published the results of a study that explored previous NTP results indicating effects on male fertility and the hematopoetic system (Web Table 1). Adult male F344 rats, 10 per group, were fed diets containing 0, 0.625, 1.25, 2.5, or 5.0% BBP for 14 days. Using actual pre-treatment body weights (200 g) and reported food intake during the 14-day dosing period, equivalent doses of 0, 447, 890, and 1,338 mg/kg bw/day were calculated for the 3 lower dose groups. Since the highest dose group actually lost weight during the study, average weight during the study was used to calculate a dose of 1,542 mg/kg bw/day. All treated rats showed a dose-related increase in relative liver and kidney weight. No histopathology or hematology changes were observed at the 447 or 890 mg/kg bw/day dose levels. However, at doses of 1,338 and 1,542 mg/kg bw/day, there were relative decreases in testes, seminal vesicle, and thymus weight noted; relative epididymis weight was reduced at the largest dose. Dose related histopathological changes in seminal vesicles, testes, and prostate were observed as was a decrease in bone cellularity at the two highest doses. Mild multifocal hepatitis and cortical lymphocytolysis were also observed at the high dose. Increases in luteinizing hormone (LH) were observed at the lowest dose tested; however, increases in follicle stimulating hormone (FSH) and decreases in testosterone were only observed at 1,338 and 1,542 mg/kg bw/day, respectively. The decreased body weight seen at the two highest doses may be due to unpalatibility of food; decreased food intake was documented. The severity of the reduced food intake and attendant weight loss precludes associating effects with BBP, or BBP and inanition, at the highest dose. The LOAEL determined from these studies is 447 mg/kg bw/day based on increases in organ weight (liver, kidney) and increased LH levels.

Three-month feeding studies were conducted in 4–6-week-old Wistar and Sprague-Dawley (SD) rats fed diets with 2,500–12,000 or 2,500–20,000 ppm BBP, respectively [Hammond, 1987 #229] (Web Table 2). Male Wistar rats (27–45 rats/sex/group) received doses of 151, 381, or 960 mg/kg bw/day; female doses were 171, 422, or 1,069 mg/kg bw/day. At the lowest dose, an increase in liver to body weight ratio was seen in both sexes. No histopathology or hematology changes were noted. At the mid-dose, decreases in body weights were noted in both sexes and increases in liver and kidney to body weight ratios were seen. Pancreatic tissues showed islet cell enlargement, vacuolization, congestion, inflammation, and minor fibrosis. Less frequently, additional pancreatic changes were observed, such as acinar cell atrophy, inflammation, and pyknotic nuclei. A decrease was observed in urinary pH in male rats only. At the highest doses tested, 960 (M) and 1,069 (F) mg/kg bw/day, hepatic necrosis and anemia were observed in addition to the effects seen at lower doses. Cecal enlargement, a finding of uncertain toxicological importance, was reported in this study. The LOEL for this study was 151 mg/kg bw/day based on weight change in the liver. The LOAEL was 381 mg/kg bw/day based on pathologic changes in liver and reduced body weight.

In this same study, Sprague-Dawley rats (10/sex/group) were tested at a dose of 0, 188, 375, 750, 1,125, or 1,500 mg/kg bw/day. Sprague-Dawley rats were less sensitive to BBP than Wistar rats, as no pancreatic, hepatic, testicular lesions, or cecal enlargement were observed. Neither were there changes in urinary pH or hematological parameters.

The NOAEL was set at 375 mg/kg bw/day and the LOAEL at 750 mg/kg bw/day based on increases in organ weight ratios for kidney (male) and liver (female) [Hammond, 1987 #229].

A 13-week inhalation study was also conducted in groups of 6–8-week-old SD rats (25/sex/group) [Hammond, 1987 #229] (Web Table 2). The rats were exposed to BBP mists (>90% of aerosol particles <10 μm) at concentrations of 51, 218, or 789 mg/m^3 for 6 hours/day, 5 days/week. Using EPA (1988) assumptions for rat body weights and daily inhalation rates, estimated exposure doses were 9.2, 39.4, and 143 mg/kg bw/day for males and 9.8, 42.0, and 152 mg/kg bw/day for females. NOAELs of 39.4 (M) and

42.0 (F) mg/kg bw/day were identified in this study. A LOAEL was determined at the highest dose tested, 143 (M) and 152 (F) mg/kg bw/day which was based on increases in liver and kidney organ to body weight changes. Serum glucose levels were also reduced at this dose in male rats only. No body weight changes or histopathological changes were observed.

The NTP [NTP, 1997 #543] reported results of a 26-week dietary exposure study in 6-week-old F344/N male rats (Web Table 3). Groups of 15 male rats were fed BBP in the diet at concentrations of 0, 300, 900, 2,800, 8,300, or 25,000 ppm for 26 weeks. The authors calculated doses of 30, 60, 180, and 550 mg/kg bw/day for four lowest exposure levels. A dose was not calculated in the highest exposure group because food intake could not be measured due to an excess scattering of feed. However, a dose of 1,650 mg/kg bw/day was estimated by CERHR based on intake levels observed in the lower dose groups. In the high-dose group, decreases in total body weight (due to decreased food intake) were observed, as were increases in relative liver and kidney weights. An increased incidence of macrocytic anemia was observed on days 30–180. The testis was determined the primary target organ based on weight, sperm concentration, and histopathological findings at the highest dose. Decreases in relative testis, absolute epididymis, and seminal vesicle weight were observed, as were atrophy of seminiferous tubules and degenerative changes in testis and epididymis. No histologic changes in other body tissues were seen at this dose. Testes from animals in the lower-dose groups were examined histologically and no effects were observed; lowered sperm counts were not seen at the 60, 180, or 550 mg/kg bw/day doses. A NOAEL was established at 180 mg/kg bw/day[1]. The LOAEL of 550 mg/kg bw/day reflects increases in mean cell hemoglobin after 60–180 days of treatment that may have an association with the macrocytic anemia observed at the next higher dose.

In a 3-month feeding study, 3 adult male and female Beagle dogs/group were fed diets with 10,000–50,000 ppm DBP (males: 400, 1,000, or 1,852 mg/kg bw/day; females: 700, 1,270, or 1,973 mg/kg bw/day calculated by authors) [Hammond, 1987 #229]. Difficulty in food palatability complicated interpretation of reduced body weights in low- and high-dose males and mid- and high-dose females. No other changes were observed for hematological or urinalysis measurements. In high-dose animals there were no histopathological effects in liver, testes or pancreas .

Chronic Exposure Studies

Two sets of chronic feeding studies have been performed by the National Toxicology Program [NTP, 1982 #542; NTP, 1997 #543].

Potential BBP carcinogenicity was examined in both B6C3FI mice and F344/N rats [NTP, 1982 #542]. Four to Five-week-old B6C3F1 mice (50/sex/group) were dosed through feed at concentrations of 0, 6,000, or 12,000 ppm for 106 weeks. Using EPA assumptions for B6C3F1 mice body weight and food intake (body weight: 0.03733 kg [M], 0.0353 kg [F],; food intake: 0.0064 kg/day [M]), dose levels of 0, 1,029, and 2,058 mg/kg bw/day and 0, 1037, and 2,074 mg/kg bw/day were calculated for males and females, respectively. No treatment-related changes in survival or neoplastic developments were seen. Dose-related decreases in body weight were seen in both male and female mice.

F344/N rats (50/sex/dose) were fed diets containing 0, 6,000, or 12,000 ppm BBP [NTP, 1982 #542] for 106 weeks. Using EPA assumptions for F344 rat body weight and food intake (M:0.380 kg, 0.030 kg/day; F:0.229 kg, 0.021 kg/day), dose levels of 0, 474, and 948 mg/kg bw/day and 0, 550, 1,100 mg/kg bw/day

[1] The NTP (1997) report stated that epididymal sperm concentration was determined for the lowest and two highest of the treated groups. CMA reports that an audit revealed the original laboratory report, that is the data source for the NTP Report, states that epididymal sperm counts were determined from the three highest dose groups. The data from the original report are used in this evaluation.

were estimated for males and females, respectively. Male rats were sacrificed 29–30 weeks into the study because of increases in premature deaths. Internal hemorrhaging was suspected as the cause of these deaths. Body weight gain and food intake were decreased in both males and females. The female mice were allowed to continue through the 106 weeks of exposure and at autopsy the females exhibited increased incidence of mononuclear cell leukemia (MNCL). Spleens were examined in the high-dose treatment group and were found to be congested and infiltrated with mononuclear cells. MNCL has been associated with spleenomegaly and sometimes hepatomegaly. No evidence of hepatomegaly was reported in these studies.

In the 2-year NTP bioassay [NTP, 1997 #543]groups of 60 male Fischer 344 rats (6-weeks-old) were fed BBP in the diet at concentrations of 0, 3,000, 6,000, or 12,000 ppm (0, 120, 240, or 500 mg/kg bw/day) and 60 females (6-weeks-old) per group were fed concentrations of 0, 6,000, 12,000, or 24,000 ppm (0, 300, 600, or 1,200 mg/kg bw/day) (Web Table 4) [NTP, 1997 #543]. After 2 years of exposure to BBP, increases in relative kidney organ weights were observed in male rats at 120 mg/kg bw/day and represented the first observable changes in this study[NTP, 1997 #543].

Dose-related increases were seen in relative kidney (at the 120 mg/kg bw/day dose), epididymis (240 mg/kg bw/day), and liver (500 mg/kg bw/day) weight in the male rats with total body weight changes in rats occurring only at the highest dose tested, 500 mg/kg bw/day. At this level, histopathological changes included renal tubule pigmentation, hepatic granulomas, and focal pancreatic hyperplasia with "some evidence" of pancreatic carcinogenicity. No testicular changes were observed; however, decreases in RBC and increases in hemoglobin were observed at 6 months into the study. There was no histologic or pathologic evidence of peroxisome proliferative effects in the liver.

Female F344/N rats exposed to BBP for 2 years showed nephropathy at the two lowest doses tested (300 and 600 mg/kg bw/day). At 1,200 mg/kg bw/day, the animals exhibited decreases in body weight and increases in liver and kidney organ to body weight ratio. They also exhibited renal tubule pigmentation (15–24 months), nephropathy, microcytic anemia (15 months), decreases in triiodothyronine, and "equivocal evidence" of pancreatic and urinary bladder carcinogenicity. Pancreatic effects may have been due to chronic stimulation of pancreatic lipase secretion.

In a parallel study at the same laboratory, BBP's ability to induce hepatic peroxisomes was evaluated in female F344/N rats [NTP, 1997 #543]. Two enzyme markers for peroxisome proliferation, palmitoyl CoA oxidase and carnitine acetyl transferase, were significantly elevated after 1 month or 1 year of exposure in animals exposed to 6,000 ppm BBP and greater (~240 mg/kg bw/day), although the level of induction was lower than that observed after a 3-week exposure to DEHP. The discussion in the NTP report highlights the fact that BBP is a mild proliferater compared to DEHP or hypolipidemic drugs such as clofibrate.

From these 2-year studies, LOAELs for non-cancer, general toxicity effects were determined at 120 and 300 mg/kg bw/day based on kidney organ weight changes in the male and nephropathy in the females. At 500 (M) and 1,200 mg/kg bw/day (F), the highest doses tested respectively, "some to equivocal evidence" of pancreatic (male and female) and urinary bladder carcinogenicity (female) was observed in rats. No testicular changes were observed at any of the doses tested; however, increases in epididymal weights were seen at the 2 highest doses (240 and 500 mg/kg bw/day). This change in epididymal weight was observed in the absence of total body weight change at the 240 mg/kg bw/day exposure dose.

2.2 Toxicokinetics

Absorption

Dermal

In a study of dermal absorption of a series of phthalate diesters [Elsisi, 1989 #126], ^{14}C-BBP (157 μmol/kg) was applied to the skin (clipped back) of male F344 rats and the area covered with a perforated cap. Absorption was estimated by the radioactivity eliminated in urine and feces over 7 days, which equaled 27% for BBP. Most of the remainder of the radioactivity was found at the site of application.

Oral

Oral administration of 5g of BBP/kg to dogs resulted in 10% absorption [Erickson, 1965 #1045]. Administration of single oral doses of 2, 20, 200, or 2,000 mg/kg to male Fischer 344 rats showed a dose-dependent increase in the fraction of dose eliminated via the feces (20% at doses from 2–200 mg/kg; 72% at 2,000 mg/kg) and a dose-dependent decrease in the fraction eliminated via the urine (75% at a dose of 2–200 mg/kg and 22% at 2,000 mg/kg), suggesting that absorption through the gut was limited at the high dose [Eigenberg, 1986 #122].

Biotransformation

Oral studies in rats indicate that absorbed BBP is rapidly metabolized to its monoester metabolites (monobutyl and monobenzyl esters) and that these monoesters are excreted or conjugated with glucuronic acid and then excreted via the urine [Eigenberg, 1986 #122; Elsisi, 1989 #126; Erickson, 1965 #1045; Mikuriya, 1988 #630]. Urinary metabolites in rats following oral administration of 3.6 mmol BBP/kg/day for 3 days indicated that 70% of the metabolites were monoesters while the remainder were monoester conjugates. The monobutyl ester is generally present in the highest amount; in one study the ratio of monobutyl to monobenzyl phthalate was 5:3 [Mikuriya, 1988 #630]. The glucuronidation pathway appears to be saturated at high doses, as noted by the decrease in the glucuronide metabolite relative to the monoester metabolites at high doses (2,000 mg/kg in rats) versus low doses (20 mg/kg in rats).

Distribution

Tissue distribution was non-specific for the small amount of dermally absorbed BBP [Elsisi, 1989 #126].

Excretion

Excretion of absorbed BBP and its metabolites is rapid, with approximately 90% eliminated in 24 hours, with approximately 80% excreted in urine and 20% in feces at low doses (2–200 mg/kg). The half-life of BBP in blood is 10 minutes. The blood half-life of the monoester metabolites of BBP is approximately 6 hours [Eigenberg, 1986 #122]. Following intravenous administration of 20 mg/kg of ^{14}C-BBP, 55% of the dose was excreted into bile while 34% was excreted in the urine [Eigenberg, 1986 #122].

Side-Chain Associated Toxicokinetics

Phenol metabolism is well known as a primary alcohol that is easily oxidized to butyric acid (n-butanoic acid) by alcohol dehydrogenase and aldehyde dehydrogenase. Further metabolism by (α-oxidation pathways converts butyric acid into acetyl-CoA conjugates in intermediary metabolism pathways with no toxicological importance [Di Carlo, 1990 #262].

2.3 Genetic Toxicity

The NTP [NTP, 1997 #543] reviewed the genetic toxicity of BBP. An increase in mutations was not observed following treatment of *Salmonella* and L5178Y mouse lymphoma cells with BBP in the presence and absence of S9 activation. BBP treatment with and without S9 activation did not result in sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells. However, induction of sister chromatid exchanges and increased chromosomal aberrations in bone marrow cells were observed following a single intraperitoneal injection of mice with 1,250–5,000 mg/kg bw/day BBP. There were no increases in sex-linked recessive lethal mutations in the germ cells of *Drosophila* fed or injected with BBP.

Subsequent to the NTP review, BBP tested negative in the L5178Y mouse lymphoma mutation assay with and without activation, and in the Balb/3t3 cell transformation assay [Barber, #893]. Ashby et al. [, 1997 #37] reported negative results in a micronucleus assay in rats. The IPCS [IPCS, 1999 #1023] review included the publication of Ashby et al and concluded: "Although the weight of evidence of genotoxicity is clearly negative, available data are inadequate to unequivocally conclude that BBP is not clastogenic. However, in the available studies, the activity has been weak and is often consistent with secondary effects of the chemical on DNA."

See Section 5.1.2 for a summary of general biological and toxicological studies.

3.0 DEVELOPMENTAL TOXICITY DATA

3.1 Human Data

Studies in humans with butyl benzyl phthalate were not found.

3.2 Experimental Animal Toxicity

Eleven complete studies and one abstract were evaluated. Two, performed through the National Toxicology Program, were standard prenatal assessment (segment II) studies of BBP administered in the diet of rats and mice. The third was an oral gavage Segment II study in rabbits. There were five studies by Ema et al. in Wistar rats where BBP was administered in the diet or by gavage. Three studies of BBP evaluated drinking water exposure to Wistar rats during gestation and lactation with assessment of adult F_1 males. One abstract evaluated BBP exposure by subcutaneous injection to two strains of male mice (B6C3F1 and CD-1) with subsequent mating to unexposed females (dominant lethal assessment).

3.2.1 Prenatal Development

A dietary study in CD (Sprague-Dawley) rats [Field, 1989 #157] involved exposure of 30 pregnant rats per group to 0, 0.5, 1.25, and 2.0% BBP (0, 420, 1,100, and 1,640 mg/kg bw/day) on gestation day (gd) 6–15. The dams were killed on gd 20, necropsied, and pups examined and evaluated (i.e., a Segment II study

design) (Web Table 5). Maternal toxicity was expressed in reduced body weights and decreased weight gain, decreased absolute feed consumption (but increased relative feed consumption in g/kg/day), increased relative liver weight (with no histopathological changes), and increased relative water intake at the top two doses. Relative kidney weights were increased at the 1,640 mg/kg bw/day dose. However, the kidneys were not examined histologically. Clinical signs of maternal toxicity, including ataxia and abnormal gait, were also observed at this dose.

At 1,640 mg/kg bw/day, there were increased resorptions and concomitant reduced numbers of live fetuses per litter, reduced fetal body weight, and increased fetal malformations. Urogenital malformations, analyzed separately, were increased; they included distended ureters and distended or absent kidneys. Other fetal malformations at the high dose were anophthalmia (missing eyes), fused or malaligned vertebrae, and fused ribs. There were increased incidences of fetal variations per litter at both the 1,100 and 1,640 mg/kg bw/day doses.

Significant developmental toxicity, including teratogenicity at 1,640 mg/kg bw/day, occurred at doses resulting in maternal toxicity. The maternal and developmental NOAELs were identified at 420 mg/kg bw/day.

Ema et al. [, 1990 #145] exposed pregnant Wistar rats, 15–18/group, to BBP in the diet at 0, 0.25, 0.5, 1.0, and 2.0% (intakes of 0, 185, 375, 654, and 974 mg/kg bw/day, respectively) on gd 0–20. Dams were killed on gd 20 and evaluated in a Segment II study design (Web Table 6). There were also pair-fed controls matched with the animals in the highest dose group tested. No dams died in any group. Maternal body weight gains and feed consumption were reduced at doses of 654 and 974 mg/kg bw/day. All dams at 974 mg/kg bw/day had fully resorbed litters. There was no treatment-related pre-implantation loss or teratogenicity. The authors concluded that the maternal NOEL was 375 mg/kg bw/day and the developmental toxicity NOEL was 654 mg/kg bw/day. The Expert Panel did not agree with the author's conclusion that the data supported the developmental toxicity NOAEL given that fetal body weights (by sex per litter) were significantly reduced and live litter size was apparently reduced (12.3 versus control value of 13.9) at 654 mg/kg bw/day. The data did support a developmental NOEL at 375 mg/kg bw/day, the same as the maternal NOEL.

In a second segment II study, Ema et al. [Ema, 1992 #137] treated 10 Wistar rats/group with BBP by gavage with 0, 500, 750, or 1,000 mg/kg bw/day on gd 7–15 (Web Table 7). Dams and fetuses were evaluated following sacrifice on gd 20. Maternal body weight gains were reduced at doses of 750 and 1,000 mg/kg bw/day, but the corrected weight gain (maternal body weight excluding the gravid uterus) was only decreased at the top dose. Food intake was reduced at all dose levels. Four dams in the high dose group died and entire litters were resorbed in the six surviving dams. Complete litter resorptions were observed in 3 of 10 dams in the 750 mg/kg bw/day group. Other effects at that dose level included increased fetal death due to postimplantation loss, reduced fetal weight, and increased external, skeletal, and internal malformations. The types of malformations consisted primarily of cleft palate, fused sternebrae, and dilated renal pelves. The maternal and fetal NOAEL was identified as 500 mg/kg bw/day.

The segment II dietary study in CD-1 mice [Price, 1990 #524] involved exposure of 30 pregnant mice per group to 0, 0.1, 0.5 and 1.25% BBP (0, 182, 910, and 2,330 mg/kg bw/day), on gd 6–15 (Web Table 8). Maternal toxicity was expressed as reduced weight gain at the highest two doses (910 and 2,330 mg/kg bw/day), and increased relative liver and kidney weights and increased relative water intake, at the latter dose. No histopathological changes were observed in liver or kidney.

Embryofetal effects included increased incidences of resorptions and late fetal deaths, with concomitant reductions in live fetuses per litter, and increased malformations (external and skeletal) at 910 and

2,330 mg/kg bw/day. Malformations included exencephaly, short tail, cardiovascular defects, fused ribs, and abnormal or fused sternebrae and vertebrae. Fetal body weight per litter was decreased and fetal variations were increased at the 2,330 mg/kg bw/day dose. As with rats, maternal and developmental toxicity was present at the highest two doses. The maternal and developmental NOAEL was 182 mg/kg bw/day.

A segment II developmental toxicity study [Monsanto, 1978 #623] was also performed in New Zealand white rabbits. The does, 17/group, were administered BBP (Santicizer 160) orally by gelatin capsule on gestation days 6–18 at 0, 3.0, or 10 mg/kg bw/day. Doses were terminated on gd 29. There was no demonstrable maternal toxicity. There was no demonstrable developmental toxicity such as effects on fetal body weight, 24-hour survival, or treatment-related external or visceral malformations. Skeletal findings in *toto* were considered equivalent across groups.

Mechanistic Studies

The Ema group has published a series of articles that focus on three issues: 1) direct vs. indirect toxicity of BBP; 2) the dose and time dependency of the prenatal effects of BBP exposure; and 3) study of the toxic properties of the two monoester metabolites of BBP.

Direct vs. indirect toxicity. In addition to the dietary study in Wistar rats previously described [Ema, 1990 #145], a follow-up study involved dietary exposure to BBP at 2.0% (974 mg/kg bw/day) on gd 0–20 with pair-fed controls (repeat), gd 0–11 or gd 11–20 [Ema, 1991 #136]. As in the earlier study, all dams exposed on gd 0–20 had fully resorbed litters. The pair-fed controls exhibited maternal weight gains comparable to the BBP group, but no treatment-related fetal malformations or resorptions were observed. Dams fed BBP on gd 0–11 also had fully resorbed litters. No increase in post-implantation loss was found in rats exposed on gd 11–20, but the fetuses in this group exhibited malformations, predominantly cleft palate and fused sternebrae. Thus, resorption does not appear to be related to decreased food consumption, but is an effect of the chemical, *per se*.

Time and dose dependency. In another dietary study using 2.0 % BBP on gd 0–7, gd 7–16, gd 16–20 [Ema, 1991 #146][Ema, 1992 #134], post-implantation loss was increased after exposure on gd 0–7 or 7–16; teratogenicity was observed (predominantly cleft palate and fused sternebrae) after exposure on gd 7–16 [Ema, 1992 #134]. Ema et al. [, 1991 #146; Ema, 1992 #137] also dosed Wistar rats by gavage with BBP in olive oil at 0, 500, 750, or 1,000 mg/kg bw/day on gd 7–16. No live fetuses were present at 1,000 mg/kg bw/day and malformations (cleft palate, fused sternebrae, dilated renal pelves) occurred at 750 mg/kg bw/day accompanied by increased *in utero* death, decreased fetal body weight, and maternal toxicity (reduced weight gain and feed consumption). At 500 mg/kg bw/day, maternal feed consumption during the exposure period was reduced, but no embryofetal effects were observed.

To further investigate the observed embryolethality and teratogenicity, Ema et al. [, 1994 #141] exposed Wistar rats to BBP in the diet at 2.0% (954 mg/kg bw/day) during gd 0–20, 0–7, 0–9, or 0–11. Pre-implantation loss was equivalent across all groups. Post-implantation loss was highest for groups treated on gd 0–11. Uterine and ovarian weights were reduced as was plasma progesterone in all groups (except ovarian weight was unaffected on gd 7). The authors suggest that the post-implantation loss in early pregnancy was mediated by reduced plasma progesterone levels from impairment in luteal function.

It appears that post-implantation death or the development of malformations is dependent upon the dose and time during gestation when the exposure occurs.

Studies on monoesters. Ema et al. evaluated the developmental toxicity of the two metabolites of BBP: mono-n-butyl phthalate (mBuP) [Ema, 1996 #143; Ema, 1995 #139; Ema, 1995 #140] and mono-n-benzyl phthalate (mBeP) [Ema, 1996 #143] when administered by gavage to Wistar rats.

Ema et al. [Ema, 1995 #140] gavaged Wistar rats with mBuP at 0, 250, 500, and 625 mg/kg bw/day on gd 7–15. Maternal toxicity was present at the top two doses, expressed as reduced body weight gains and reduced feed consumption. At these doses there were also significant increases in post-implantation loss/litter, and decreases in live fetuses/litter and fetal body weight per litter. Fetal malformations were also increased at these doses, with cleft palate, deformed vertebral column and dilated renal pelves the predominant findings.

Ema et al. [Ema, 1996 #143] followed-up with evaluation of stage specificity studies. Wistar rats were dosed with mBuP at 0, 500, 625, or 750 mg/kg bw/day on gd 7–9, 10–12, or 13–15. Embryolethality was increased at all doses for all dosing intervals. No teratogenicity was observed from the gd 10–12 dosing interval. Increased incidences of fetal external malformations were present in the groups treated with 500 and 750 mg/kg bw/day on gd 7–9 and 13–15. Increased skeletal malformations were observed in groups treated with 500, 625, and 750 mg/kg bw/day on gd 7–9 and with 625 and 750 mg/kg bw/day on gd 13–15. Deformed cervical vertebrae were predominant in groups treated on gd 7–9. Cleft palate and fused sternebrae were observed in groups treated on gd 13–15. These results are consistent with the findings for DBP and BBP, and imply that mBuP (and/or subsequent metabolites) may account for the developmental toxicity (embryolethality and malformations) for both DBP and BBP.

Ema et al. [Ema, 1996 #143] also administered mBeP by gavage at 0, 250, 313, 375, 438, and 500 mg/kg bw/day to pregnant Wistar rats on gd 7–15. Decreased maternal weight gain during dosing was present at doses from 313 to 500 mg/kg bw/day, and reduced feed consumption was present from 250 to 500 mg/kg bw/day. Increased postimplantation loss was present at 438 and 500 mg/kg bw/day. Increased incidences of fetal external malformations were present at 438 and 500 mg/kg bw/day, skeletal malformations were present at 313–500 mg/kg bw/day, and visceral ("internal") malformations at 375–500 mg/kg bw/day. The most common fetal findings were effects on cervical and thoracic vertebrae, ribs, and kidney (dilated renal pelves at 375 and 438 mg/kg bw/day, and hypoplasia of the kidney at 500 mg/kg bw/day).

These studies establish a maternal and developmental NOAEL for mBuP of 250 mg/kg bw/day. For mBeP, no maternal NOAEL was identified (effects were observed at 250 mg/kg bw/day); the developmental NOAEL was 250 mg/kg bw/day under the conditions of the study. The finding of fetal kidney effects at 375–500 mg/kg bw/day for mBeP is of concern since the CD rat study [Field, 1989 #157] also found fetal kidney malformations at the high dietary dose (1,640 mg/kg bw/day) and the kidneys is a known target organ in adult rats. Cervical ribs are also of concern (due to their rarity).

An additional study [Ema, 1995 #139] compared effects of BBP and DBP administered by gavage at 0, 750, 1,000 or 1,250 mg/kg bw/day on gd 7–9, 10–12, or 13–15. Increased postimplantation loss was observed for both compounds at all doses from all exposure periods. Malformations were observed in groups treated with both phthalate esters at ≥750 mg/kg bw/day on gd 7–9 (vertebral column and ribs) and on gd 13–15 (cleft palate and fused sternebrae). No malformations were observed with either compound at any dose when they were administered on gd 10–12. The authors concluded that "the similarity in dependence of gestational days of treatment on the manifestations of developmental toxicity and on the spectrum of fetal malformations caused by BBP and DBP suggests that they may act by the same mechanism, possibly via a common metabolite of these two parent compounds."

3.2.2 Postnatal Development

Imajima et al. [, 1997 #862] gavaged pregnant Wistar-King A (WKA) rats with monobutyl phthalate ester (mBuP) in sesame oil at 0 or 300 mg/kg bw/day on gd 15–18 (equivalent to approximately 1,000 mg/kg bw/day). Male offspring were evaluated on gd 20 and on postnatal day (pnd) 30–40 to determine the position of the testes. In control males, all testes were located in the lower abdomen on gd 20 (19 pups, 3 litters) and had descended into the scrotum on pnd 30–40 (15 pups, 3 litters). In stark contrast, in males exposed *in utero* to mBuP, on gd 20 all testes were located high in the abdominal cavity (15 pups, 3 litters) with significantly higher testes ascent. On pnd 30–40, mBuP exposed males exhibited cryptorchidism (22 of 26 pups, 5 litters with uni- or bi-lateral undescended testes); 87% of the undescended testes were in the abdominal cavity, the remaining 13% were located at the external inguinal ring. Testis descent is under androgenic control; the authors suggest that phthalate esters may interfere with FSH stimulation of cAMP accumulation in Sertoli cells, resulting in the reduced secretion of mullerian inhibiting substance, a putative mediator in transabdominal migration of the testis.

3.2.3 Postnatal Function

Sharpe et al. [, 1995 #696] reported on adult male offspring from Wistar rat dams exposed 2 weeks prior to mating, and during gestation and lactation, to BBP (in ethanol) in the drinking water at 1,000 μg/L (Web Table 9). This study combined data from two separate exposures to the same dams to assess the effects of BBP. At weaning, male offspring were reared to adulthood, with no further BBP exposure and assessed for reproductive effects. Maternal BBP intake was calculated by weighing water bottles for 48-hour intervals, On pnd 1–2, 10–12, and 20–21, BBP intake was estimated at 126, 274, and 336 μg/kg bw/day (the latter 2 measurements were confounded by pups drinking the treated water). Male offspring had significantly smaller testes, but no effects on body, kidney, or ventral prostate weights. Testicular morphology and seminiferous epithelial tubule cross-sections were unaffected, but the authors believe there was reduced daily sperm production.

Ashby et al. [, 1997 #37] attempted to replicate the Sharpe et al. [, 1995 #696] findings with larger group sizes and better control and characterization of the dosing material. They exposed 18 AP (Wistar) rats during prebreed, gestation, and lactation to 1,000 μg/L BBP in the drinking water and assessed the F_1 male offspring as adults (Web Table 10). They found no effects of BBP exposure on any endpoints assessed, including testis weights, daily sperm production, caudal epididymal sperm count, accessory sex organ weights, or relative incidence of gonadotrophs (FSH-positive cells) in the pituitary for male or female offspring. This study employed only a single dose level.

Another replication of the Sharpe et al. [Sharpe, 1995 #696] study was attempted by TNO [TNO, 1998 #611] (Web Table 11). They exposed Wistar rats, 28 females/group, to BBP in the drinking water at 100, 1,000, and 3,000 μg/L during premating, gestation, and lactation periods. Doses to dams were estimated at 0, 12, 140, and 385 mg/kg bw/day. No effects were observed on mating index, female fecundity or fertility, or on postimplantation loss in the parental generation. The study failed to reproduce any effects on F_1 male reproductive organ weights or daily sperm production rates when the F_1 offspring reached adulthood. Preputial separation in males and estrous cyclicity in females were also unaffected by BBP treatment.

There was a significant decrease in pup survival in the mid- and high-dose BBP groups (and the DES-positive control), with the number of pups missing or found dead between pnd 1 and 4 being 2, 30, 29, and 39 in the 0, 12, 140 and 385 μg/kg bw/day BBP groups and the DES groups, respectively. According to the authors, the values for BBP were not statistically significant on a per litter basis. This study, in turn, was

followed up within the same laboratory using a control and mid- and high-dose BBP groups with comparable treatment regime and dose levels to those indicated above. In this case, the number of pups found missing or dead for pnd 1–4 was 29, 11, and 42 in the control, mid-, and high-dose groups, respectively. Thus, values were significantly decreased compared to control at the mid-dose and again significantly increased compared to control at the high dose level. Again, statistical significance was reported by the authors as not being achieved on a per litter basis. Interestingly, significant effects on pup survival were reproduced at the highest dose level of BBP used (~ 350 μg/kg/d during gestation). There were no effects on sexual malformations on either sex of F_1 offspring, and no effects on testis weight or daily sperm production. A decreased number of normal epididymal sperm was found in the low dose group, and was not considered treatment related. Epididymal sperm motility was normal.

Bayer [, 1998 #955] repeated the study conducted by TNO [, 1998 #612] to determine if the increased perinatal pup death was reproducible (Web Table 12). Because the study was not intended to reproduce the original findings by Sharpe et al. [, 1995 #696], it did not include an evaluation of pup testes weight and sperm counts. In the study by Bayer [, 1998 #955], 28 Wistar rats/group were exposed to 0, 1, or 3 ppm BBP through drinking or diet (0, 1,000, or 3,000 μg BBP/L water or kg food) from 2 weeks prior to mating throughout the gestation and lactation period. Pups were evaluated for viability and weight gain until sacrifice on pnd 21. Unlike the TNO study, there was no effect on perinatal pup survival during gd 1–4. The authors did note increased post-implantation loss in dams treated through drinking water (8.6, 10.3, and 13.3%) and feed (5.7, 11.7, and 7.9%). Because the increases were not statistically significant and were within historical control values, they were not considered to be treatment-related. It was concluded that BBP treatment had no effect on litter size, pup survival, and pup weight gain.

As reported in an abstract, Parks et al. [, 1999 #986] dosed Sprague-Dawley rats by gavage with 750 mg/kg bw/day of BBP, DEHP, or corn oil (vehicle) from gd 14 through pnd 3. On pnd 2, anogenital distance (AGD), and testis weight were measured. Testes weights and AGD were significantly decreased and the incidence of retained areolae on pnd 13 was increased for both DEHP- and BBP-exposed male pups. Testicular testosterone production was evaluated *in vitro* from male fetuses/pups on gd 17, 18, 19, and pnd 2; it was significantly reduced on gd 18 and 19 (maximal) and on pnd 2 for DEHP but not for BBP. The antiandrogenic effects observed in male rats from perinatal exposure to DEHP (and other phthalates) may be due to reduced androgen production in fetal Leydig cells; the authors suggest that the testis is the target organ for perinatal phthalate exposure. It is not yet known whether these effects are mediated via direct action of phthalates on the fetal Leydig cell or through alterations of Sertoli cell paracrine secretions.

According to an abstract, a dominant lethal study was performed [Bishop, 1987 #62] on B6C3F1 and CD-1 male mice administered BBP by subcutaneous injections on days 1, 5, and 10 of the study at doses equivalent to 400–600, 1,280–1,840, and 3200–4560 mg/kg bw/day (triethylene melamine was the positive control). The males were then paired with untreated females every 4 days through day 49; female uterine contents were evaluated on gd 17. BBP did not affect prenatal deaths or fertility in either strain at any dose.

See section 5.1.3 for a summary of developmental toxicity.

4.0 REPRODUCTIVE TOXICITY

4.1 Human Data

There were no human data available for review by the Expert Panel.

4.2 Experimental Animal Toxicity

Six studies were reviewed in the evaluation of the reproductive toxicity of BBP. None of the studies available are definitive and no multigeneration-reproduction study has been published for this phthalate ester. Three studies measured reproductive performance. One other has reported claims of low level effects of BBP on reproductive development, but these effects have not been reproduced by two separate laboratories.

A study for the assessment of the reproductive toxicity of BBP was reported by Piersma [Piersma, 1995 #514] (Web Table 13). This was a standard reproductive toxicity screen conducted according to the OECD 421 protocol and provides useful indications as to major toxic effects. Male and female WU rats (10/sex/group), 10–11 weeks old at the start of exposure, were gavaged for 14 days with BBP in corn oil at dose levels of 0, 250, 500, or 1,000 mg/kg bw/day, and then paired (1:1) and allowed to mate for a maximum of 14 days while dosing continued. Once evidence of mating was observed, the animals were separated. Males continued to be dosed daily, and were then killed and necropsied after a total dosage period of 29 days. Reproductive organs were removed and placed in Bouins fixative. Dosing of females continued until pnd 6 after which the females were killed and necropsied and ovaries and uteri examined. Pups were counted, sexed, weighed, and examined for external malformations on pnd 1 and 6 and then killed.

Body weight gain for the F_0 males was reduced at the high dose level (by 21%), whereas the body weight gain of the F_0 females was increased in the second week of dosing (12 g/week compared to 4 g/week for the controls). During pregnancy, the body weight gain of the dams was significantly reduced at the high dose level (by 40%). The number of animals achieving a pregnancy was 9, 8, 7, and 4 (of 10) in the 0, 250, 500, and 1,000 mg/kg bw/day groups, respectively. Postnatal pup mortality was not different across dose groups, but the average litter size at birth was 9.4, 11.4, 8.4, and 1.5 in the 0, 250, 500, and 1,000 mg/kg bw/day groups, respectively with statistical significance achieved at the highest dose. Absolute pup weight was significantly reduced at birth in the high- (29%) and mid-dose (7%) groups. Testicular degeneration accompanied by interstitial cell hyperplasia was significantly increased in the high-dose group F_0 males. Ovary structure was not affected by treatment.

In Piersma et al. [, 1995 #514] the high-dose group had lower fertility (decreased numbers of litters and decreased numbers pups per litter) in the F_0 generation with marked histopathology in the testes, but not in the ovaries. F_1 pup weight was reduced at birth in the mid- and high-dose groups with a NOAEL of 250 mg/kg bw/day. Our confidence in the quality of the study is moderate to high; because of the design limitations, such as a lack of measures in the F_1 generation, there is uncertainty that these doses correctly represent the true NOAEL.

A one-generation reproduction study (OECD guideline 415) was performed in Wistar rats that were mated twice and produced 2 litters [TNO, 1993 #610] (Web Table 14). BBP was administered in the diet at levels of 0, 0.2, 0.4, and 0.8% to male and female rats for 10 and 2 weeks prior to the first mating, respectively. Seven to thirteen days after the first litter was weaned (at pnd 21) the study was repeated with the same rats. Average doses to males during the premating period were estimated by authors at 0, 108, 206, or 418 mg/kg bw/day. Average female doses during the premating, gestation, and lactation periods were estimated at 0, 106, 217, or 446 mg/kg bw/day; 0, 116, 235, or 458; and 0, 252, 580, or 1078 mg/kg bw/day, respectively. There were no treatment-related clinical signs or mortality. There were periods of reduced body weight or weight change in females in the high-dose group during gestation and lactation in each of the two litterings. A decrease in food consumption during the gd 0–14 period in both matings was considered a substance-

related effect. A slight decrease in the number of treated females with litters observed in the first mating was not observed in the second mating. Mean pup weight was slightly decreased in the high-dose group during lactation; this decrease reached statistical significance at pnd 21 in the second litter. The authors attributed the day-21 finding to direct consumption of BBP in diet after pnd 14. All standard reproductive indices were within normal ranges. At necropsy, tissues from male and female reproductive organs were collected and fixed in 4% buffered formalin. Microscopic examination of hematoxylin- and eosin-stained slides from these tissues was performed for rats that were in either the control or high-dose groups. The examination revealed that liver and reproductive tissues were normal. The authors concluded that the NOAEL for reproductive performance was 418 mg/kg bw/day in males, and 446–1,078 mg/kg bw/day in females with the parental NOAEL being 206 mg/kg bw/day in males and 217−580 mg/kg bw/day in females.

The NTP [NTP, 1997 #543] (Web Table 15) described a 10-week modified mating study. Male F344 rats, 6-weeks-old at the commencement of the study, were exposed to BBP (15/group) in the diet at levels of 0, 300, 2,800, or 25,000 ppm for 10 weeks (which delivered approximately 0, 20, 200, 2,200 mg/kg bw/day) and then allowed to recover for 2 days. The rats were then housed individually with 2 untreated females during a 7–day mating period and females were removed on the first day of a vaginal plug or sperm detection. Females were necropsied on gd 13. After the mating period, 10 and 11 days after receiving the last dose in feed, the males were necropsied and a full histological examination made at 0 and 25,000 ppm only. However, the testis and epididymis, seminal vesicles, and prostate were examined in all groups. The fixative used to preserve testes was not indicated. Epididymal sperm analysis was also performed on the males; sperm samples were collected for evaluation at the end of the study.

Mean body weights of the high-dose males were 71% of control values at the end of the study, representing a significant reduction. Food consumption differences between the control and high-dose groups at the end of the study were only modestly decreased with treatment when proportionality to body weight is considered. Liver and thymus to body weight ratio were increased in the high-dose group, whereas absolute and relative testis and prostate weights were reduced. There was marked degeneration in the testis and epididymis at this dose. One animal in the low-dose group had marked testicular atrophy and others had fewer sperm in the epididymis. Epididymal sperm concentration was reduced at all dose levels: 87, 70, and 0.1 % of control at the 20, 200, and 2,200 mg/kg bw/day groups, respectively. Other sperm parameters (motility, morphology) were not measured in the high dose group due to the absence of sperm; sperm motility and morphology were not different from controls in the other treatment groups. Although 10 of 30 females mated to high-dose males were-sperm positive during the mating trial, none were pregnant at necropsy. The pregnancy measures of the two lower dose groups levels were similar to control values.

In the NTP study, the high-dose group had a high rate of infertility (decreased numbers of pregnancies) with marked histopathology in the testes and epididymides and a lowered sperm count. Effects in the middle-dose group (200 mg/kg bw/day) were restricted to a significant reduction in sperm count. The NOAEL for male reproductive toxicity was 20 mg/kg bw/day based on the reduction in epididymal sperm count at the middle dose. It must also be noted that this may not correctly represent the true NOAEL, because of the lack of measures to assess effects in females, and there was no assessment of reproductive system in the F_1 generation. However it was subsequently noted that sperm counts might have been affected by a shorter recovery period from the time between mating to necropsy in the 200 mg/kg bw/day group compared to the other dose groups [Barter, 1999 #951].

For example, Judd et al. provide the most recent example of significant body of literature showing that sperm levels in the cauda epididymis are significantly reduced by ejaculation; in some cases counts are reduced to <50% of control values [Judd, 1997 #1046; Ratnasooriya, 1987 #1047]. Because epididymal sperm counts in rats have been found to require at least 4 days to return to normal after mating

[Ruangsomboon, 1985 #1048] and 13 of the 15 rats in the 200 mg/kg bw/day group were killed less than 4 days after the detection of a vaginal plug in their mats, while only 7 control males were killed in this same period, the reduction in sperm count in the 200 mg/kg bw/day group in this 10-week study must be considered at least questionable. Additionally, an expert reviewing methods of sperm analysis stated that at least a week should transpire between mating and necropsy in order to avoid ejaculation-induced confounding of sperm count data [Seed, 1996 #1049].

In parallel with the 10-week modified-mating study [NTP, 1997 #543], a 26-week sub-chronic study was performed where male F344 rats received BBP in the diet at doses of 0, 300, 900, 2,800, 8,300, and 25,000 ppm (0, 30, 60, 188, 550, and 1,650 mg/kg bw/day). The results of this study are presented in the section on General Toxicity [Web Table 3]. While a mating sequence was not part of the 26-week-study design, all other protocol parameters associated with male effects (organ weights, tissues for microscopic evaluation, and epididymal spermatozoal parameters) were identical to the NTP 10-week study. A comparison of results shows similarity in effects on body weight gain, organ weights, histopathological findings, and sperm motility. Interestingly, while sperm concentration in the 200 mg/kg bw/day group was reduced by 30% in the 10-week study, the values for the 550 mg/kg bw/day group in the 26-week study were not reduced. All other measures at this dose were similar to controls. Results of the other two doses, compared to their contemporary controls, were similar.

A study by Agarwal et al. [Agarwal, 1985 #10] (Web Table 1) examined the effect of doses of BBP on the male reproductive system of adult rats. Fischer F344 rats (10 males per group) aged 12–13 weeks were administered BBP at levels of 0, 0.625, 1.25, 2.5, and 5% (20, 447, 890, 1,338, and 1,542 mg/kg bw/day) in the diet for 14 days and killed on day 15. At necropsy, a range of tissues were taken for weighing and histological examination, including the testes and accessory sex organs. Testes were fixed with Bouin's solution. The general results are presented in Section 2.2 with the reproductive effects discussed below.

Overall body weight gain was significantly reduced at the two highest dose levels as was food consumption. Absolute weights of testes and epididymides, prostate, and seminal vesicles were reduced in the 2.5 and 5% groups in a dose dependent manner. These reductions were accompanied by largely degenerative (atrophic) changes in these organs. Plasma testosterone was reduced significantly at the highest dose level with elevations in FSH at the two highest dose levels.

In Agarwal et al. [, 1985 #10], the largest two doses produced significant weight and histological changes to the testis and accessory sex glands accompanied by changes in circulating FSH and LH levels. A NOAEL for reproductive toxicity in this 14-day study in adult male F344 rats was 1.25 % (890 mg/kg bw/day) BBP in the diet. Expert Panel confidence in the quality of the study is moderate as, within design limitations, the study is well conducted and reported. Our confidence is low that these dose levels correctly represent the true NOAEL because of the short exposure time and lack of measures in younger animals or the F_1 generation.

Studies on postnatal male fertility, with animals exposed indirectly through maternal consumption, as reported by Sharpe et al. [, 1995 #696], and subsequent publications by Ashby et al. [, 1997 #37] and TNO [, 1998 #612] that failed to reproduce the original findings are presented and discussed in Section 3.2.

Mode of Action.

Hormonal activity. BBP has been shown to bind to the estrogen receptor (ER) [Jobling, 1995 #300; Zacharewski, 1998 #741]. The relative binding affinity is approximately 10,000–100,000 times less than 17ß-estradiol (E2). BBP also induces weak activity in *in vitro* estrogen-mediated gene expression assays in

mammalian cell transfection experiments at 10 M, the highest concentration examined [Zacharewski, 1998 #741]. In a yeast assay of estrogen-mediated gene expression, the potency of BBP was $1x10^6$–$5x10^7$ that of E2, but its metabolites mBuP and mBeP demonstrated no estrogenic activity [Harris, 1997 #831]. However, no effects on uterine wet weight and vaginal epithelial cell cornification were observed in 10 Sprague-Dawley rats/group gavaged with 20, 200, and 2,000 mg/kg bw/day for 4 days [Zacharewski, 1998 #741].

See Section 5.1.4 for summary of Reproductive Toxicity studies.

5.0 DATA SUMMARY & INTEGRATION

5.1 Summary

5.1.1 Human Exposure

BBP is used in PVC construction materials and to manufacture PVC automotive materials and food conveyor belts [CMA, 1999 #825]. There appears to be no significant use of BBP in toys or medical equipment. It is believed that only negligible amounts of BBP are present in air due to its low volatility and a limited number of air monitoring studies support this view. Therefore, inhalation exposure of the general population is insignificant [IPCS, 1999 #1023]. However, inhalation exposure to BBP in manufacturing facilities has been estimated at 86 μg/kg bw/day [CMA, 1999 #825]. Exposure through contact of BBP-containing materials with skin is negligible due to the relatively slow absorption through skin [IPCS, 1999 #1023; Elsisi, 1989 #126; Scott, 1987 #644]. The Expert Panel concluded that consumption of food containing trace levels of BBP is the only significant source of exposure to the general population. Based on a survey of Canadian foods, IPCS [IPCS, 1999 #1023] estimated that exposure of adults to BBP is 2 μg/kg bw/day and that exposure levels in children could be up to 3-fold higher. MAFF estimated the BBP exposure of adults through diet at 0.11–0.29 μg/kg bw/day and exposure of infants through formula at 0.1–0.2 μg/kg bw/day. In all exposure estimates, it was evident that exposure to the general population, including children, is well below 10 μg/kg bw/day.

Utility of Exposure Data for CERHR Evaluation. BBP exposures resulting from food intake have been estimated by two authoritative sources. Limitations in the data set are that one agency used data that were 12–15 years old and may not reflect current exposure. Further, the data were collected in Europe and Canada and may not accurately reflect U.S. patterns.

5.1.2 General Biological and Toxicological Data

Toxicity. There are no human data on the general toxicity of BBP. BBP is not acutely toxic by the oral or dermal route as evidenced by the LD_{50} value exceeding 2 g/kg bw [IPCS, 1999 #1023]. In repeat-dose studies, mice were less sensitive to toxic effects than were rats. Dietary studies of up to 2-years duration in $B6C3F_1$ mice showed dose-related reductions in body weight at doses of 1,029 mg/kg bw/day and higher [NTP, 1982 #542]. There was no clinical or histological evidence of toxicity in tissues, including male and female reproductive organs. Male dogs also appear to be less sensitive than rats because oral doses up to 1,852 mg/kg bw/day for 90 days resulted in reduced body weight but produced no histopathological effects

in testes or liver [Hammond, 1987 #229]. Several subchronic and chronic dietary studies in rats reported consistent adverse effects on body weight and in kidney, liver, and testes [Hammond, 1987 #229; Agarwal, 1985 #10; NTP, 1997 #543]. Effect levels were similar between subchronic and chronic studies. The earliest response was an increase in kidney, or liver and kidney, to body weight ratio and was observed at doses of 120 mg/kg bw/day and higher. Histological changes in liver were observed in some studies at doses of 960 mg/kg bw/day and greater and changes in kidneys were observed in the chronic study at doses of 500 (M) – 1,200 (F) mg/kg bw/day. Anemia was observed at doses of 500 mg/kg bw/day and greater. The pancreas may also be a target organ, as pancreatic lesions were reported in a subchronic study at 381 mg/kg bw/day. Lesions in testes, seminal vesicles, epididymis, and/or prostate were noted after exposure of rats to 1,338 mg/kg bw/day or greater. In an inhalation study in rats, increases in liver and kidney weights were reported at the maximum doses of 143 (M) – 152 (F) mg/kg bw/day [Hammond, 1987 #229].

Carcinogenicity. NTP 2-year dietary studies found no evidence of carcinogenicity in B6C3F1 mice and only a marginal increase in leukemia in F344 female rats [NTP, 1982 #542]. In a subsequent NTP study in F344 rats, there was some evidence of carcinogenicity in males exposed to 500 mg/kg bw/day based on an increased incidence of pancreatic acinar adenomas and carcinomas [NTP, 1997 #543]. There was equivocal evidence of carcinogenicity in females exposed to 1,200 mg/kg bw/day based on marginal-increases of pancreatic acinar cell adenoma and transitional epithelial papilloma of the urinary bladder. BBP is considered to induce weak peroxisome proliferation in rats.

Toxicokinetics. There are no data from studies in humans. BBP is rapidly absorbed (at least 75% at doses of 2–200 mg/kg) in orally-dosed rats; this dropped to 22% at 2,000 mg/kg, suggesting saturation at high doses [Eigenberg, 1986 #122]. BBP is absorbed slowly through the skin (27% in 7 days) of rats [Elsisi, 1989 #126]. Eigenberg et al. also reported that BBP is rapidly metabolized to monobutyl and monobenzyl esters; by analogy to other phthalate esters, this probably occurs by pancreatic lipase and esterases in the small intestine. The monobutyl ester is usually present in higher amounts (5:3) than is the monobenzyl ester [IPCS, 1999 #1023]. These monoesters are excreted or conjugated with glucuronic acid and then excreted in the urine [Erickson, 1965 #1045; Eigenberg, 1986 #122; Elsisi, 1989 #126; Mikuriya, 1988 #630]. The glucuronidation pathway appears to be saturated at high doses, as noted by the decrease in the glucuronide metabolite relative to the monoester metabolites at high doses (2,000 mg/kg in rats) versus low doses (20 mg/kg in rats). There is no evidence of accumulation in tissues. Excretion of the absorbed BBP and its metabolites is rapid with approximately 90% elimination in 24 hours with approximately 80% in urine and 20% in feces at low doses (2–200 mg/kg). The half-life of BBP in blood is 10 minutes; the blood half-life of the monoester metabolites of BBP is approximately 6 hours.

Genetic toxicity. A recent review by the International Program on Chemical Safety [IPCS, 1999 #1023] stated: "Although the weight of evidence of genotoxicity is clearly negative, available data are inadequate to unequivocally conclude that BBP is not clastogenic. However, in the available studies, the activity has been weak and is often consistent with secondary effects of the chemical on DNA."

Utility of Data to the CERHR Evaluation. The oral subchronic studies in rats and mice are adequate for the evaluation of general toxicity induced by BBP. Some studies were conducted according to GLP standards and relevant exposure routes were utilized. Adult rodents and dogs were tested for BBP-induced testicular lesions. The examination of hepatic effects was adequate and included a limited evaluation of peroxisomal proliferation in rats.

There are acceptable toxicokinetic data for BBP, consisting of absorption, distribution, metabolism, and excretion data following oral and dermal exposure in the rat.

Table 2: Summaries of NOAELs and LOAELs and Major Effects in Oral General Toxicity Studies

Protocol and BBP Doses (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day) and Effects	Major effects at higher doses
14-day repeat dose dietary study in adult male Fischer 344 rats. 10 rats/group. Doses 0, 447, 890, 1,338, or 1,542 mg/kg bw/day. [Agarwal, 1985 #10]	None	447 ↑LH. ↑Liver and kidney weight.	↓Testes, seminal vesicle, epididymis. ↓Thymus weight. Histopathological effects in testes, seminal vesicles, and prostate. ↑LH and FSH ↓Testosterone. ↑Liver and kidney weight. Histological changes in liver and thymus. ↓Bone marrow cellularity.
3-month repeat dose dietary study in Wistar rats. 4–6 weeks old at start of study. 27–45 rats/sex/group. Doses – M: 0, 151, 381, 960 mg/kg bw/day. F: 0, 171, 422, 1,069 mg/kg bw/day. [Hammond, 1987 #229]	None but LOEL= M: 151; F: 171 ↑ Liver weight.	M: 381; F: 422 ↑Liver and kidney weight. Pancreatic lesions. ↓Urine pH (M).	↑Liver weight. Liver lesions. Pancreatic lesions. Anemia. ↓Urine pH (M). **No** testicular lesions.
90-day repeat dose dietary study in adult Beagles. 3/sex/group. Doses – M: 0, 400, 1,000, 1,852 mg/kg bw/day. F: 0, 700, 1,270, 1,973 mg/kg bw/day. [Hammond, 1987 #229]	M: None F: 700	M: 400 F: 1,270 Decreased body weight.	No histological effects in liver or testes.
26-week dietary study in adult male Fischer 344 rats. 6-weeks-old at start of study. 15 rats/group. Doses – 0, 30, 60, 180, 550, 1650. [NTP, 1997 #543]	180	550 ↑Liver weight. ↑Hemoglobin.	↓Testes, seminal vesicle, and epididymis weight. Lesions in testes and epididymis. ↓ Sperm counts. ↑ Liver and kidney weight. Anemia.
2-year dietary study in Fischer 344/N rats. 6-weeks-old at start of study. 60 rats/sex/group. Doses – M: 0, 120, 240, 500 mg/kg bw/day; F: 0, 300, 600, or 1,200 mg/kg bw/day. [NTP, 1997 #543]	None	M: 120; F: 300 ↑Kidney weight (M). Nephropathy (F).	↑ Liver weight. ↑ Kidney weight. Nephropathy. Anemia. ↓ Thyroid hormone (F). Some evidence of pancreatic cancer (M). Equivocal evidence of urinary bladder and pancreatic cancer (F). No testicular lesions.
2-year dietary study in B6C3F1 mice. 4–5 weeks old at start of study. 50 mice/sex/group. Doses – M: 0, 1,029, 2,058 mg/kg bw/day; F: 0, 1,037, 2,074 mg/kg bw/day. [NTP, 1982 #542]	None	M: 1,029; F: 1,037 ↓Weight gain.	↓ Weight gain. No changes in survival or neoplasm development.

5.1.3 Developmental Toxicity

Studies of prenatal development consistently show BBP to be embryolethal and teratogenic following exposure to high oral doses in rats and mice on gd 6 or 7–15. The incidence of these effects is dependent on dose and developmental age. A maternal and developmental NOAEL in CD-1 mice was 182 mg/kg bw/day [Price, 1990 #524]. The Expert Panel noted that there was wide spacing between the NOAEL and the LOAEL of 910 mg/kg bw/day. Effects at the LOAEL and higher doses included increased resorptions and late fetal deaths, reduced live fetuses per litter, and increased external and skeletal malformations.

Developmental NOAELs in Sprague Dawley and Wistar rats ranged from 420 to 500 mg/kg bw/day, respectively [Ema, 1992 #137]. Effects at doses greater than or equal to 750 mg/kg bw/day included increased prenatal mortality, reduced fetal growth, and increased fetal variations and skeletal, visceral, and external malformations. Extending the exposure period to gd 0–20 in Wistar rats resulted in a developmental NOAEL (375 mg/kg bw/day) similar to that seen when dosing was confined to gd 6–15. An oral prenatal study in rabbits revealed no maternal or developmental toxicity at doses of 10 mg/kg bw/day; however, utility of the results is limited since no maximum tolerated dose was established [Monsanto, 1978 #623].

Using a prenatal study design similar to that used with BBP [Ema, 1992 #137], the monoesters (mbuP and mBeP) were investigated [Ema, 1995 #140; Ema, 1996 #132]. The developmental toxicity observed with the monoesters was qualitatively similar to that produced by BBP. These data suggest that both monoesters can contribute to the developmental toxicity associated with BBP. Differences in the doses selected for study do not permit a close quantitative comparison of dose-effect between the two monoesters or with BBP. A rat study, using a mBuP dose of 1,000 mg/kg bw/day, reported a subsequent interference with testicular migration and descent [Imajima, 1997 #862].

Studies in rats indicated that prenatal effects are directly related to the chemical and are not due to decreased food consumption [Ema, 1991 #136]. The mechanism of action for resorption has been proposed as reduced circulating progesterone due to impaired luteal function [Ema, 1994 #141].

The effect of low dose exposure during mating, gestation, and lactation on postnatal maturation in males has been studied. Sharpe et al. [Sharpe, 1995 #696] observed reduced testicular weights and sperm production in the offspring of Wistar rats exposed to 1 mg BBP/L drinking water throughout mating, gestation, and lactation. This laboratory subsequently reported unexplained fluctuation in testicular weight of control rats [Sharpe, 1998 #697]. The original findings of Sharpe et al. were not replicated in equivalent experiments performed by two other laboratories with doses as high as 3 mg/L [TNO, 1998 #611; Ashby, 1997 #37]. An increase in pup mortality reported by TNO [TNO, 1998 #611] at 1 and 3 mg/L was not observed in the studies by Sharp [Sharpe, 1995 #696] and Ashby [Ashby, 1997 #37] or in a subsequent study by Bayer [Bayer, 1998 #955] at doses up to 3 mg/L. The Expert Panel recommends that the reproductive effects in F_1 males reported by Sharpe et al. [Sharpe, 1995 #696] not be used in assessing of the reproductive toxicity of BBP. The bases for the recommendation are: 1) lack of dose-response data (e.g., a single-dose study); 2) the original laboratory has had difficulty in replicating their original findings; and 3) two other respected laboratories have been unable to replicate the effects. The Expert Panel also notes that the increased perinatal pup mortality observed by TNO [TNO, 1998 #611] was not replicated in other laboratories.

Table 3: Summaries of NOAELs and LOAELs and Major Effects in Developmental Toxicity Studies

Protocol and Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)		Developmental Effects Observed at Higher Dose Levels
		Maternal	Developmental	
Prenatal dietary study in Sprague-Dawley rats. 30 per group received 0, 420, 1,100, or 1,640 mg/kg bw/day on gd 6–15. Dams and pups examined late in gestation. [Field, 1989 #157]	Maternal: 420 Developmental: 420	1,100 ↓ Body weight gain. ↑ Liver weight.	1,100 ↑ Variations.	↑ Prenatal mortality. ↓ Fetal weight. ↑ Visceral, skeletal, and external malformations. ↑ Variations.
Prenatal dietary study in Wistar rats. 15–18/group received 0, 185, 375, 654, or 974 mg/kg bw/day on gd 0–20. Dams and pups examined late in gestation. [Ema, 1990 #145]	Maternal: 375 Developmental: 375	654 ↓ Weight gain.	654 ↓ Fetal weights.	Complete prenatal mortality.
Prenatal gavage studies conducted in Wistar rats. 10/group received BBP 0, 500, 750, or 1,000 mg/kg bw/day (0, 1.60, 2.40, 3.20 mmol/kg bw/day) on gd 7–15. Dams and pups examined late in gestation. [Ema, 1992 #137]	Maternal: 500 Developmental: 500 (1.60 mmol)	750 (2.40 mmol) ↓ Weight gain.	750 (2.40 mmol) ↑ Prenatal mortality. ↓ Fetal weight. ↑ Internal, external, and skeletal variations.	Complete prenatal mortality.
The same study was conducted with **mBuP** in rats/group that received 0, 250, 500, or 625 mg/kg bw/day (0, 1.13, 2.25, 2.81 mmol/kg bw/day). [Ema, 1995 #140]	Maternal: 250 Developmental: 250 (1.13 mmol)	500 (2.25 mmol) ↓ Weight gain.	500 (2.25 mmol) ↑ Prenatal mortality. ↓ Fetal weight. ↑ External and skeletal malformations. ↑ Visceral variations.	↑ Prenatal mortality. ↓ Fetal weight. ↑ External and skeletal malformations. ↑ Visceral variations.
The same study was conducted with **mBeP** in 10–13 rats/group that received 0, 250, 313, 375, 438, or 500 mg/kg bw/day (0, 0.976, 1.22, 1.46 mmol/kg bw/day). [Ema, 1996 #132]	Maternal: None Developmental: 250 (0.976 mmol)	250 (0.976) ↓ Food intake.	313 (1.22) ↑ Skeletal malformations.	↑ Prenatal mortality. ↑ Internal, external, and skeletal malformations.
Prenatal dietary study in CD-1 mice. 30 per group received 0, 182, 910, or 2,330 mg/kg bw/day on gd 6–15. Dams and pups examined late in gestation. [Price, 1990 #524]	Maternal: 182 Developmental: 182	910 ↓ Weight gain.	910 ↑ Prenatal mortality. ↑ Visceral, skeletal, and external malformations.	↓ Fetal weight. ↑ Prenatal mortality. ↑ Visceral, skeletal, and external malformations. ↑ Variations.

Utility of the Data for CERHR Evaluation. The data in rats and mice are adequate for a prenatal assessment of fetal growth, lethality, and teratogenicity. One study examined prenatal effects following exposure during

late pregnancy. None of the studies included a postnatal evaluation of androgen-regulated effects (e.g., nipple retention, testicular descent, or preputial separation) that were the most sensitive indicators of developmental toxicity with DBP. DBP and BBP share a common monoester metabolite. Prenatal studies with BBP monoesters (mBuP and mBeP) were sufficient to determine that metabolites contribute to developmental toxicity. Because of differences in doses administered to the mouse and rats, it is not possible to compare sensitivity between the two species.

5.1.4 Reproductive Toxicity

There are no data in humans that assess the reproductive effects of BBP. All experimental animal studies that assess reproduction have been performed in the rat.

Male rat reproductive toxicity. Male reproductive performance was evaluated in three rat studies by the oral route of exposure [Piersma, 1995 #514; TNO, 1993 #610; NTP, 1997 #543]. There were no effects in reproductive performance in 10 rats exposed to up to 500 mg/kg bw/day by gavage for 2 weeks prior to mating. Decreased fertility and testicular histopathology were seen at 1,000 mg/kg [Piersma, 1995 #514]. No adverse effects were noted in rats exposed through diet with up to 418 mg/kg bw/day from 10 weeks prior to mating until the birth of a second litter; reduced fertility was observed at doses of 1,000 mg/kg bw/day [TNO, 1993 #610]. Reduced sperm counts were noted in rats exposed to 200 mg/kg bw/day through diet for 10 weeks, but reproductive performance was not affected [NTP, 1997 #543]. In a subchronic dietary study by the same group, no effects on sperm counts were reported following a 26-week exposure to a dose of 550 mg/kg bw/day, but sperm counts were reduced following treatment with 1,650 mg/kg bw/day [NTP, 1997 #543]. For the 10-week study, it was subsequently noted that sperm counts may have been affected by a shorter recovery period from the time between mating to necropsy in the 200 mg/kg bw/day group compared to the other dose groups, and that the results from the 10-week study are therefore questionable [Barter, 1999 #951]. Because the known required recovery time for cauda epididymal sperm counts after ejaculation [Ruangsomboon, 1985 #1048] was not reached for most of the animals in the 200 mg/kg bw/day group, and because a higher dose group in a subsequent study had sperm counts the same as the control group, the Expert Panel has considers the reduced sperm levels at 200 mg/kg bw/day to be sufficiently questionable that these data are not relied upon for setting a NOAEL or LOAEL. Histopathology of male reproductive organs has also been examined in subchronic and chronic rodent studies by the oral route and the lowest dose to produce testicular lesions in rats fed diets with 1,338 mg/kg bw/day [Agarwal, 1985 #10]. The reproductive organs of male mice were unaffected at dietary doses up to 2,058 mg/kg bw/day and testes of dogs were not affected at dietary doses up to 1,852 mg/kg bw/day by the oral route. The Expert Panel selected a reproductive NOAEL of 500 mg/kg bw/day for adult male rats. There is uncertainty as to the dose that is without effect on the developing male reproductive tract. The Expert Panel noted that a primary BBP metabolite, mBuP, is likely the active toxicant in DBP studies where exposure during *in utero* development or during the neonatal period of life led to reproductive effects [Mylchreest, 2000 #962]. The existing studies with BBP did not critically examine pups during the sensitive postnatal phases of life. It is probable that such studies would likely result in a lower NOAEL.

Female reproductive toxicity. In a reproduction and toxicity screening study, decreases in the number of females conceiving and in the number of live pups per litter were observed at an oral gavage dose of 1,000 mg/kg. Clear testicular effects in males suggest that the effect may be due in part to toxicity in the male [Piersma, 1995 #514]. A dose of 500 mg/kg was a NOAEL for female fertility in this study. No effects on implantation, reproductive organ morphology, fertility, or fecundity were seen in a 1-generation reproductive toxicity study in Wistar rats that received the highest dose of 446–1,078 mg/kg bw/day in diet [TNO, 1993 #610].

Mode of Action

Hormonal activity. BBP has been shown to bind to the estrogen receptor (ER) [Jobling, 1995 #300; Zacharewski, 1998 #741]. The relative binding affinity is approximately 10,000–100,000 times less than 17β-estradiol (E2). It also induces weak activity in *in vitro* estrogen-mediated gene expression assays at 1,000 μM, the highest concentration examined, but its metabolites mBuP and mBeP demonstrated no estrogenic activity [Harris, 1997 #831]. However, no effects on uterine wet weight and vaginal epithelial cell cornification were observed in Sprague-Dawley rats gavaged with 20, 200, and 2,000 mg/kg body weight.

Table 4: Summaries of NOAELs, LOAELs, and Major Effects in Reproductive Toxicity Studies

Protocol & Study	NOAEL (mg/kg bw/day)	Reproductive LOAEL (mg/kg bw/day) and effects	Systemic LOAEL (mg/kg bw/day)and Effects	Reproductive Effects at Higher Doses
One generation reproductive screening assay in WU rats. 10 pairs/group received 0, 250, 500, or 1,000 mg/kg bw/day by gavage from 2 weeks prior to mating until pnd 6 [Piersma, 1995 #514]*	Reproductive: 500 Systemic: 500	1000 ↓Fertility. Testicular lesions. ↓Litter size.	1000 ↓Weight gain.	No higher doses in study.
One generation dietary reproductive toxicity assay in Wistar rats with 12 males and 24 females/group. Males were treated 10 weeks prior to mating with 0, 108, 206, or 418 mg/kg bw/day. Females were treated from 2 weeks prior to mating (0, 106, 217, or 446 mg/kg bw/day), through gestation (0, 116, 235, or 458 mg/kg bw/day) and lactation (0, 252, 580, or 1078 mg/kg bw/day). [TNO, 1993 #610]*	Reproductive: 418 (M); 446–1,078 (F) Systemic: 206 (M); 217–580 (F)	No structural or functional effects at any dose.	418 (M); 446–1,078 (F) ↓Weight gain (F) ↑Liver and kidney weight.	No higher doses in study.
One generation modified mating study in male F344 rats. 15 males/group treated with DBP through diet at 0, 20, 200, or 2,200 mg/kg bw/day for 10 weeks and then mated with untreated females. [NTP, 1997 #543]	Reproductive: 20 Systemic: 200	200 ↓ Sperm counts.	2,200 ↓ Weight gain. ↑ Liver weight. Anemia	↓ Fertility. Testicular and epididymal lesions. ↓ Sperm counts. ↓ Testes and prostate weight.

Utility of Data to the CERHR evaluation. The data in rats are adequate for an assessment of reproductive toxicity in adults. Studies are available that evaluate both structure and reproductive function. However, the offspring were not evaluated in these studies and there is therefore no information on transgenerational effects. In studies with DBP, a phthalate that is also metabolized to mBuP, male rats exposed while *in utero* and during lactation were most sensitive to DBP-induced effects on reproductive structure and function [Wine, 1997 #690]. Therefore, the most sensitive age for reproductive toxicity was not addressed for BBP.

5.2 Integrated Evaluation

BBP is primarily used in PVC construction materials and is also used to manufacture PVC automotive materials and food conveyor belts. Exposure of the general population through inhalation is negligible due to the low volatility of BBP. However, inhalation exposure to BBP in manufacturing facilities has been estimated at 86 μg/kg bw/day. Exposure through contact with skin is negligible due to the relatively slow absorption through skin. The Expert Panel has concluded that consumption of food containing trace levels of BBP is a significant source of exposure to the general population. Estimates based on BBP levels in Canadian and UK foods indicate that exposure to the general population, including children, is below 10 μg/kg bw/day.

There are no human toxicokinetic or toxicity studies for BBP. Studies in rats demonstrate that orally-administered BBP is rapidly converted to the monoester metabolites (mBuP and mBeP) within the gut. At low doses (<2 mg/kg), about 80% of the administered dose is metabolized and the metabolites are absorbed into systemic circulation. The remainder of the dose is excreted in feces unchanged. Absorbed metabolites are glucuronidated and rapidly excreted in urine with no evidence of accumulation. The Expert Panel believes the toxicokinetic studies in rats to be relevant to human exposure of BBP through food.

Prenatal exposure studies in rats and mice have indicated that oral exposure on gd 6 or 7–15 to high doses of BBP (> 500 mg/kg bw/day) result in reduced fetal growth, prenatal mortality, and visceral, skeletal, and external malformations. NOAELs of 182 mg/kg bw/day and 500 mg/kg bw/day were identified for mice and rats, respectively; however, a comparison of sensitivity between species is not possible due to variations in doses administered. Oral prenatal studies with BBP metabolites (mBuP and mBeP) have demonstrated qualitatively similar results to BBP and suggest that the metabolites are associated with the developmental toxicity. None of the studies examined the postnatal effects on the male reproductive system. This is of concern because standard prenatal studies do not detect effects such as testicular lesions, retained nipples, and delayed preputial separation. Such effects have been observed with DBP, the monoester metabolite of which is the same as one of the metabolites of BBP. Therefore, the Expert Panel is not confident in the NOAELs obtained from the existing BBP developmental studies, and believes that lower NOAELs may be observed in studies with late gestational exposure and complete postnatal examination of the male reproductive system.

The data indicate that BBP is a reproductive toxicant in adult male rats as evidenced by testicular lesions, reduced sperm counts, and increased infertility following exposure to oral doses exceeding the NOAEL of 500 mg/kg bw/day. Effects on the reproductive system of adult female rats are less certain. There were no reproductive effects in female rats exposed orally to 446–1,078 mg/kg bw/day from 2 weeks prior to mating through lactation. However, in a second study, the number of females conceiving litters was reduced following exposure to 1,000 mg/kg bw/day by gavage. The data do not permit clear delineation as to whether this was male- or female-related although clear evidence of testicular toxicity was seen. The Expert Panel notes that the database does not allow for a complete evaluation of reproductive effects due to the lack of a multigeneration study.

The Expert Panel believes the database is sufficient to judge that oral exposure to BBP can cause reproductive toxicity in rats and developmental toxicity in rats and mice. These data are assumed to be relevant to humans. We are not confident that the lowest dose at which developmental toxicity, specifically effects on the developing male reproductive tract, has been established.

Tentative Research needs / Data Gaps for BBP

1) Multigeneration study. (There is a priority need for a multigenerational study that evaluates effects on reproductive development, fertility, and reproductive system structure with continuous exposure across multiple generations. Female reproductive effects need to be evaluated explicitly.)
2) *In utero* exposure with postnatal developmental assessments
3) Clarify potential effects of BBP on sperm parameters in rats.
4) Need for toxicokinetics studies in primates.

Human studies: No studies of humans were found. Occupationally exposed cohorts might be located but these would be of limited usefulness if the major exposure source is food.

6.0 REFERENCES

This is an early draft and the reference list may not be complete

1. CMA. Comments of the Chemical Manufacturers Association phthalate esters panel in response to request for public input on seven phthalate esters. FR Doc. 99-9484. Washington, DC: Chemical Manufacturers Association, 1999.
2. IPCS. Concise international chemical assessment document 17 - Butyl benzyl phthalate. Geneva, Switzerland:WHO, 1999.
3. MAFF. Food surveillance information sheet - Phthalates in infant formulae - follow-up survey. Joint Food Safety and Standards Group, vol 1999:MAFF - UK, 1998;13.
4. MAFF. Phthalates in infant formulae. Joint food safety and standards group food surveillance information sheet, vol 1999:MAFF - UK, 1996;7.
5. MAFF. Phthalates in food. Joint food safety and standards group food surveillance information sheet, vol 1999:MAFF - UK, 1996;9.
6. MAFF. Phthalates in infant formulae. Food surveillance information sheet 168: Joint Food Safety and Standards Group, 1998.
7. DHHS. Phthalates in infant formula - assignment summary: Public Health Service, 1996.
8. Rastogi SC. Gas chromatographic analysis of phthalate esters in plastic toys. Chromatographia 47:724-726(1998).
9. Elsisi AE, Carter DE, Sipes IG. Dermal absorption of phthalate diesters in rats. Fundam Appl Toxicol 12:70-77(1989).
10. Scott RC, Dugard PH, Ramsey JD, Rhodes C. In vitro absorption of some o-phthalate diesters through human and rat skin. Environ Health Perspect 74:223-227(1987).
11. Hammond BG, Levinskas GJ, Robinson EC, Johannsen FR. A review of the subchronic toxicity of butyl benzyl phthalate. Toxicol Ind Health 3:79-97(1987).
12. Agarwal DK, Maronpot RR, Lamb J, IV, Kluwe WM. Adverse effects of butyl benzyl phthalate on the reproductive and hematopoietic systems of male rats. Toxicology 35:189-206(1985).
13. NTP NTP-ò. Toxicology and carcinogenesis studies of butyl benzyl phthalate (CAS no. 85-68-7). in F344/N rats (feed studies). Rep nr. NTP TR 458, NIH Publication No. 97-3374: U.S. Department of Health and Human Services, Public Health Service, National Institute of Health, 1997.
14. NTP NTP-ò. Carcinogenesis bioassay of butyl benzyl phthalate (CAS no. 85-68-7) in F344/N rats and B6C3F1 mice (feed study). Rep nr. NTP-80-25, NIH Publication No. 82-1769.: U.S. Department of Health and Human Services, Public Health Service, National Institute of Health., 1982.
15. Erickson NG. The metabolism of diphenyl phthalate and butylbenzyl phthalate in the beagle dog. Dissertation Abstracts 26:3014-3015(1965).

16. Eigenberg DA, Bozigian HP, Carter DE, Sipes EG. Distribution, excretion, and metabolism of butylbenzyl phthalate in the rat. J Toxicol Environ Health 17:445-456(1986).
17. Mikuriya H, Ikemoto I, Tanaken A. Urinary metabolites contributing to the testicular damage induced by butylbenzyl phthalate. Jikeikai Med J 35:403(1988).
18. Di Carlo F. Structure-activity relationships (SAR) and structure-metabolism relationships (SMR) affecting the teratogenicity of carboxylic acids. Drug Metab Rev 22:441-449(1990).
19. Barber ED, Cifone M, Rundell J, Przygoda R, Astill BD, Moran E, Mulholland A, Robinson E, Schneider B. Results in the L5178Y mouse lymphoma, and the *in vitro* transformation of Balb/3T3 cell assays for eight phthalate esters. J Appl Toxicol in press:39.
20. Ashby J, Tinwell H, Lefevre PA, Odum J, Paton D, Millward SW, Tittensor S, Brooks AN. Normal sexual development of rats exposed to butylbenzyl phthalate from conception to weaning. Regul Toxicol Pharmacol 56:102-118(1997).
21. Field EA, Price CJ, Marr MC, Myers CB. Developmental toxicity evaluation of butyl benzyl phthalate (CAS No. 85-68-7) administered in feed to CD rats on gestational days 6 to 15 NTP-89-246. Research Triangle Park: National Toxicology Program, 1989.
22. Ema M, Murai T, Itami R, Kawasaki H. Evaluation of the teratogenic potential of the plasticizer butyl benzyl phthalate in rats. J Appl Toxicol 10:339-43(1990).
23. Ema M, Itami T, Kawasaki H. Teratogenic evaluation of butyl benzyl phthalate in rats by gastric entubation. Toxicol Lett 61:1-7(1992).
24. Price CJ, Field EA, Marr MC, Myers CB. Final report on the developmental toxicity of butyl benzyl phthalate (CAS No. 85-68-7) in CD-1-Swiss mice. NTP-90-114. Research Triangle Park: National Toxicology Program, National Institute of Environmental Health Sciences, 1990.
25. Monsanto MC--. Teratogenic study with sanitizer 160 in albino rabbits IBT No. 8580-09859. Decatur: Monsanto Company, 1978.
26. Ema M, Itami T, Kawasaki H. Evaluation of the embryolethality of butyl benzyl phthalate by conventional and pair-feeding studies in rats. J Appl Toxicol 11:39-42(1991).
27. Ema T, Itami T, Kawasaki H. Teratogenicity of butyl benzyl phthalate in rats. Teratology 44:166(1991).
28. Ema M, Itami T, Kawasaki H. Effect of period of exposure on the developmental toxicity of butyl benzyl phthalate in rats. J Appl Toxicol 12:57-61(1992).
29. Ema M, Kurosaka R, Amano H, Ogawa Y. Embryolethality of butyl benzyl phthalate during early pregnancy in rats. Reprod Toxicol 8:231-236(1994).
30. Ema M, Kurosaka R, Harazono A, Amano H, Ogawa Y. Phase specificity of developmental toxicity after oral administration of mono-n-butyl phthalate in rats. Arch Environ Contam Toxicol 31:170-176(1996).
31. Ema M, Kurosaka R, Amano H, Ogawa Y. Comparative developmental toxicity of n-butyl benzyl phthalate and di-n-butyl phthalate in rats. Arch Environ Contam Toxicol 28:223-228(1995).
32. Ema M, Kurosaka R, Amano H, Ogawa Y. Developmental toxicity evaluation of mono-n-butyl phthalate in rats. Toxicol Lett 78:101-106(1995).
33. Imajima T, Shono T, Zakaria O, Suita S. Prenatal phthalate causes cryptorchidism postnatally by inducing transabdominal ascent of the testis in fetal rats. J Pediatr Surg 32:18-21(1997).
34. Sharpe RM, Fisher JS, Millar MM, Jobling S, Sumpter JP. Gestational and lactational expsoure of rats to xenoestrogens results in reduced testicualr size and sperm production. Environ Health Perspect 103:1136-1143(1995).
35. TNO NaFRI. Oral developmental reproduction study with butyl benzyl phthalate in Wistar rats. Volume 1 of 3: European Council for Plasticizers and Intermediates, 1998.
36. Bayer AG. Butyl benzyl phthalate (BBP) - Developmental reproduction study in Wistar rats with application in the diet or drinking water 28215: Bayer AG, Institute of Toxicology, Carcinogenicity and Genotoxicity, 1998.
37. TNO NaFRI. Oral developmental reproduction study with buty benzyl phthalate in Wistar rats TNO Report V98.408 final.: Waalkens-Berendsen Ir DH. TNO Nutrition and Food Institute, 1998.
38. Parks LG, Ostby JS, Lambright CR, Abbott BD, Gray LE, Jr. Perinatal butyl benzyl phthalate (BBP) and bis(2-ethylhexyl) phthalate (DEHP) exposures induce antiandrogenic effects in Sprague-Dawley (SD) rats. Biol Reprod 60:153(1999).
39. Bishop JB, Teaf CM, Bhoosan B. Assessment of fetal death rate among in utero progeny of B6C3F1 and CD-1 mice after subcutaneous injections of males with butyl benzyl phthalate (BBP). Environ Mutagen 9:15(1987).

40. Piersma AH, Verhoef A, Dortant PM. Evaluation of the OECD 421 reproductive toxicity screening test protocol using butyl benzyl phthalate. Toxicology 99:191-197(1995).
41. TNO NaFRI. Dietary one-generation reproduction study with butyl benzyl phthalate in rats: Monsanto, 1993.
42. Barter RA. Correspondence to R. Chapin concerning Solutia's review of NTP Report 458, 1997, 1999.
43. Judd JE, Berndtson WE, Castro ACS. Extragonadal sperm reserves, sperm depletion rates, numbers of sperm per mating, and fertility with successive matings by intact or unilaterally vasectomized rats. J Androl 18:698-707(1997).
44. Ratnasooriya WD, Wadsworth RM. Effect of mating on sperm distribution in the reproductive tract of the male rat. Gamete Research 17:261-266(1987).
45. Ruangsomboon D, Visutakul P. Effect of mating on sperm numbers and weight of the epididymis. Contraception 32:217-221(1985).
46. Seed J, Chapin RE, Clegg ED, Dostal LA, Foote RH, Hurtt ME, Klinefelter GR, Makris SL, Perreault SD, Schrader SM, Seyler D, Sprando R, Treinen KA, Veeramachaneni DNR, Wise LD. Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: A consensus report. Reprod Toxicol 10:237-244(1996).
47. Jobling S, Reynolds T, White R, Parker MG, Sumpter JP. A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ Health Perspect 103:582-587(1995).
48. Zacharewski TR, Meek MD, Clemons JH, Wu ZF, Fielden MR, Matthews JB. Examination of the invitro and in vivo estrogenic activities of eight commercial phthalate esters. Toxicol Sci 46:282-293(1998).
49. Harris CA, Henttu P, Parker MG, Sumpter JP. The estrogenic activity of phthalate esters in vitro. Environ Health Perspect 1997 105:802-811(1997).
50. Ema M, Harazono A, Miyawaki E, Ogawa Y. Developmental toxicity of mono-n-benzyl phthalate, one of the major metabolites of the plasticizer n-butyl benzyl phthalate, in rats. Toxicol Lett 86:19-25(1996).
51. Sharpe RM, Turner KJ. Endocrine disruptors and testis development. Environ Health Perspect 106:A221(1998).
52. Mylchreest E, Wallace DG, Cattley RC, Foster P. Dose-dependent alterations in Androgen-regulated male reproductive development in rats exposed to di(n-butyl)phthalate during late gestation. Toxicol Sci(2000).
53. Wine R, Li L-H, Barnes LH, Gulati DK, Chapin RE. Reproductive toxicity of di-n-butyl phthalate in a continuous breeding protocol in Sprague-Dawley rats. Environ Health Perspect 105:102-107(1997).